Amycolatopsis marina sp. nov., an actinomycete isolated from an ocean sediment

Jiang Bian,1,2,3† Yan Li,4† Jian Wang,1,2,3† Fu-Hang Song,1 Mei Liu,1 Huan-Qin Dai,1 Biao Ren,1,3 Hong Gao,1 Xinling Hu,1 Zhi-Heng Liu,1 Wen-Jun Li2,4 and Li-Xin Zhang1,2,5,6

1Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China
2South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, PR China
3Graduate University of the Chinese Academy of Sciences, Beijing 100049, PR China
4Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, PR China
5Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510663, PR China
6SynerZ Pharmaceuticals Inc., Lexington, MA 02421, USA

A Gram-positive, aerobic, non-motile actinobacterium, designated strain Ms392A\textsuperscript{T}, was isolated from an ocean-sediment sample collected from the South China Sea. The isolate contained chemical markers that supported chemotaxonomic assignment to the genus Amycolatopsis. On the basis of an analysis of 16S rRNA gene sequence similarities, strain Ms392A\textsuperscript{T} represents a novel subclade within the genus Amycolatopsis, with Amycolatopsis palatopharyngis 1BDZ\textsuperscript{T} as its closest phylogenetic neighbour (99.4 % similarity). However, DNA–DNA hybridization demonstrated that strain Ms392A\textsuperscript{T} was distinct from A. palatopharyngis AS 4.1729\textsuperscript{T} (48.6 % relatedness). The polyphasic analysis demonstrated that the ocean isolate can be clearly distinguished from recognized species of the genus Amycolatopsis. Therefore, strain Ms392A\textsuperscript{T} represents a novel species of the genus Amycolatopsis, for which the name Amycolatopsis marina sp. nov. is proposed. The type strain is Ms392A\textsuperscript{T} (=CGMCC 4.3568\textsuperscript{T} =NBRC 104263\textsuperscript{T}).

The genus Amycolatopsis, classified as belonging to the family Pseudonocardiaeae (Embley et al., 1988; Warwick et al., 1994), was proposed by Lechevalier et al. (1986) for aerobic, amylolate, nocardioform actinomycetes and is well defined as a result of chemotaxonomic characterization (Lechevalier et al., 1986; Henssen et al., 1987; Mertz & Yao, 1993; Yassin et al., 1993) and phylogenetic analyses based on the comparison of 16S rRNA gene sequences (Embley et al., 1988; Warwick et al., 1994). The members of the genus Amycolatopsis are Gram-positive, non-acid-fast, non-motile actinomycetes that form branched vegetative hyphae that undergo fragmentation into rod-like and squarish elements. In addition, they are represented chemotaxonomically by the following features: wall chemotype IV (meso-diaminopimelic acid, arabinose and galactose in cell-wall hydrolysates), a tetrahydrogenated menaquinone with nine isoprene units [MK-9(H\textsubscript{4})] as the major menaquinone, a phospholipid pattern of type II sensu Lechevalier et al. (1977) (phosphatidylethanolamine as a diagnostic phospholipid), fatty acid profiles that include complex mixtures of saturated and branched-chain acids and the absence of mycolic acids. The members of this genus have DNA G+C contents in the range 66–73 mol%.

At the time of writing, the genus Amycolatopsis comprises 38 recognized species, most of which were isolated from various terrestrial environments (Goodfellow et al., 2001; Kim et al., 2002; Saintpierre-Bonaccio et al., 2005; Lee et al., 2006; Tan et al., 2006a; Groth et al., 2007; Carlsohn et al., 2007) or clinical material (Labeleda et al., 2003; Huang et al., 2004). On the basis of chemotaxonomic and morphological markers (Kim & Goodfellow, 1999) and analyses using genus-specific oligonucleotide primers (Tan et al., 2006b), Amycolatopsis strains were separated from members of the other genera classified within the family Pseudonocardiaeae. Recently, there has been an explosion of information about novel bioactive compounds isolated from members of the genus Amycolatopsis (Demain & Zhang, 2005; Zhang et al., 2005). In an effort to explore the
relatively un tapped potential of members of this genus and investigate potential applications for their secondary metabolites (Zhang et al., 2007), we attempted to isolate and identify strains from the South China Sea.

Strain Ms392A\textsuperscript{T} was isolated using the following procedure. Fresh deep-ocean sediment samples were collected in the South China Sea and kept at 4 °C for isolation as soon as possible. Serial dilutions of sample suspensions were transferred onto the selective isolation medium (SM1; Tan et al., 2006b) for the genus Amycolatopsis and incubated at 28 °C for 4 weeks.

Strain Ms392A\textsuperscript{T} was cultivated on ISP 2, ISP 3, ISP 4 and ISP 5 media (Shirling & Gottlieb, 1966) at 28 °C. Spore chains were observed for colonies grown using the coverslip technique of Kawato & Shinobu (1959). Morphological characteristics were examined by using light microscopy (CX41; Olympus) and scanning electron microscopy (S-570; Hitachi). Morphological features were observed on ISP 2 and ISP 4 media at 28 °C. The phenotypic properties of the isolate were consistent with its classification within the genus Amycolatopsis. The whitish aerial mycelium, which was produced only on ISP 2 and ISP 4, formed rod-like mycelial fragments. No diffusion pigments were produced on any of the media tested.

Physiological and biochemical characteristics of strain Ms398A\textsuperscript{T} are given in Table 1 and in the species description. Carbohydrate utilization was tested using ISP 9 (Shirling & Gottlieb, 1966) as the basal medium with filter-sterilized compounds at a final concentration of 1 % (w/v). Urease activity was determined by checking for a colour change in Bacto urea broth (Difco). The production of H\textsubscript{2}S was tested on peptone iron agar (Difco). Nitrate reduction, gelatin liquefaction and degradation of elastin and starch were examined by using previously described methods (MacFaddin, 1980). Decomposition of adenine, hypoxanthine, casein, DL-tyrosine and xanthine was examined by using the methods of Gordon et al. (1974). Antibiotic susceptibility was investigated as described by Groth et al. (2004), using antibiotic discs (Himedia). Growth over a range of temperatures (4–60 °C), pH values and NaCl concentrations was determined on ISP 2 medium. The pH range and optimum for growth and the tolerance of NaCl were examined as described by Tang et al. (2003). Catalase activity was determined by assessing bubble production in 3 % (v/v) H\textsubscript{2}O\textsubscript{2}, and oxidase activity was determined using a 1 % (w/v) solution of tetramethyl-p-phenylenediamine (Kovács, 1956).

The procedures used for the identification of cell-wall amino acids and sugars in whole-cell hydrolysates were those described by Stanec & Roberts (1974). Menaquinones were extracted by using the method of Collins et al. (1977) and were analysed by means of HPLC, as described by Tamaoka et al. (1983). Polar lipids were extracted as described by Minnikin et al. (1979) and identified by using two-dimensional TLC and spraying with specific reagents (Collins & Jones, 1980). Biomass for

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A. marina sp. nov. Ms392A\textsuperscript{T}</th>
<th>A. palato pharyngis AS 4.1729\textsuperscript{T}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utilization as sole carbon source of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+)-d-Arabinose</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td>(+)-L-Rhamnose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>(+)-Maltose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>(+)-Raffinose</td>
<td>–</td>
<td>W</td>
</tr>
<tr>
<td>Decomposition of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l-Lysine</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>W</td>
<td>–</td>
</tr>
<tr>
<td>Production of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Urease</td>
<td>–</td>
<td>W</td>
</tr>
<tr>
<td>Nitrate reductase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Drug susceptibility/resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifampicin</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Growth at/with:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 °C</td>
<td>W</td>
<td>–</td>
</tr>
<tr>
<td>12 % NaCl</td>
<td>W</td>
<td>–</td>
</tr>
<tr>
<td>pH 10.0</td>
<td>–</td>
<td>W</td>
</tr>
</tbody>
</table>

Table 1. Differential phenotypic characteristics of strain Ms392A\textsuperscript{T} and its closest phylogenetic neighbour, A. palato pharyngis AS 4.1729\textsuperscript{T}

Data were taken from this study or from Huang et al. (2004). +, positive; w, weakly positive; –, negative; r, resistant; s, sensitive.

Quantitative fatty acid analysis of strain Ms398A\textsuperscript{T} was prepared by scraping growth from TSB agar plates that had been incubated for 7 days at 28 °C. Fatty acids were extracted, methylated and analysed using the MIDI (Microbial Identification) system. The cell-wall diamino acid in the peptidoglycan layer of strain Ms392A\textsuperscript{T} was meso-diaminopimelic acid, the major sugars in the cell wall were arabinose and galactose (cell-wall chemotype IV according to Lechevalier & Lechevalier, 1980) and the predominant isoprenoid quinones were tetrahydrogenated menaquinones with eight and nine isoprene units. The phospholipids included diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylglycerol and phosphatidyl ethanolamine mannoside. The major fatty acids were iso-C\textsubscript{15} : 0 (40.35 %) and iso-C\textsubscript{16} : 0 2-OH (11.42 %). Others fatty acids that occurred in smaller amounts were C\textsubscript{17} : 1 cis\textsubscript{9} (8.61 %), C\textsubscript{16} : 1 cis\textsubscript{9} (7.79 %), C\textsubscript{16} : 0 (7.08 %), C\textsubscript{17} : 0 (4.89 %), iso-C\textsubscript{17} : 0 (3.46 %), C\textsubscript{15} : 0 (2.91 %), iso-C\textsubscript{16} : 0 H (2.66 %), antiso-C\textsubscript{17} : 0 (2.28 %), C\textsubscript{15} : 1 B (1.63 %), iso-C\textsubscript{15} : 0 (1.50 %), 10-methyl C\textsubscript{16} : 0 (1.39 %), C\textsubscript{18} : 0 10-enoic (0.71 %), iso-C\textsubscript{17} : 0 (0.53 %), C\textsubscript{16} : 1 w9c (0.40 %), C\textsubscript{18} : 1 w9c (0.38 %) and C\textsubscript{17} : 1 iso (0.34 %).
with *Amycolatopsis palatopharyngis* 1BDZ\(^\text{T}\) within a separate cluster. The 16S rRNA gene sequence similarities between strain Ms392A\(^\text{T}\) and *Amycolatopsis* species with validly published names were below 97.0\%, except for *A. palatopharyngis* 1BDZ\(^\text{T}\), which showed 99.4\% similarity (corresponding to 9 differences over 1427 locations).

To determine whether strain Ms392A\(^\text{T}\) represents a distinct species of the genus *Amycolatopsis*, DNA–DNA hybridizations were performed by applying the method of He *et al.* (2005) with five replications for each sample. Strain Ms392A\(^\text{T}\) displayed low DNA–DNA reassociation with *A. palatopharyngis* AS 4.1729\(^\text{T}\) (mean value 48.6\%). The result is far below the cut-off point recommended for the circumscription of bacterial genomic species by Wayne *et al.* (1987). The G+C content of the DNA was determined by using the HPLC method (Mesbah *et al.*, 1989) and a mean value of 70.1 mol\% was obtained.

Features that serve to differentiate strain Ms392A\(^\text{T}\) from its closest phylogenetic neighbour, *A. palatopharyngis* AS 4.1729\(^\text{T}\), are shown in Table 1. Thus, in conclusion, genotypic, chemotaxonomic and phenotypic data demonstrate that strain Ms392A\(^\text{T}\) represents a novel species of the genus *Amycolatopsis*, for which the name *Amycolatopsis marina* sp. nov. is proposed.

![Fig. 1](http://ijs.sgmjournals.org) Neighbour-joining phylogenetic tree, based on almost-complete 16S rRNA gene sequences, showing the position of strain Ms392A\(^\text{T}\) within the radiation of the genus *Amycolatopsis*. Bootstrap percentages (based on 1000 resamplings) are shown at nodes. Bar, 0.02 substitutions per nucleotide position.
Description of Amycolatopsis marina sp. nov.

Amycolatopsis marina (ma.r‘i.na. L. fem. adj. marina of the sea, marine).

Cells are Gram-positive, aerobic and non-motile and produce white aerial mycelium sparsely on ISP 2 agar medium. The branched yellow to yellow–brown substrate mycelium fragments into rod-like elements. No diffusion in the medium. The branched yellow to yellow–brown substrate cells are Gram-positive, aerobic and non-motile and collected in the South China Sea.

On: Wed, 09 Jan 2019 10:12:12


Acknowledgements

We are grateful to Yu-Guang Zhou and Yu-Hua Xin (China General Microbiological Culture Collection Center, Beijing, PR China) for technical support. This work was supported, in part, by a grant from the National 863 Project (2006AA09Z402 and 2007AA09Z443), the Chinese Academy of Sciences Innovation Projects (KSCXZ-WY-G-013) and the 973 Project (2007CB707802). L.-X. Z. received funding from the Hundred Talents Program and W.-J. L. was supported by the Program for New Century Excellent Talents in University.

References


